pUC8-2

Vector IG Sequence Link :

General : phagemid ds-DNA 2695 BP

Functions : (cloning)(transcription)(mutagenesis)(sequencing)

- **Selection :** (color blue/white)
- **Copy Number :**
- **Hosts :** (E.coli JM83)(E.coli JM105)(E.coli JM109)(E.coli NM522)

Suppliers : ()

Misc.Comments : These data and their annotation were supplied to GenBank by Will Gilbert under the auspices of the GenBank Currator Program. Assembled from pUC8 and pUV81a by F. Pfeiffer, MPI, Martinsried The pUC vectors contain the beta-galactosidase gene under the control of the lac-promoter and lac-operator. Thus they are usable as inducible expression vectors. To allow insertion of genes in any reading frame, the polylinker region was redesigned to contain the same restriction sites in all three reading frames: Trio '8' is pUC8, pUC8-1 and pUC8-2. Trio '9' is pUC9, pUC9-1 and pUC9-2.

- **Parents :** (pUC8)(pUC92b)
- **Siblings :** (pUC8-1)(pUC9-1)(pUC9-2)
- **Descendents :** ()

NCBI ENTREZ Link :

Return to Vector Homepage

The enzymes of beta-oxidation

As loss of either peroxisomal or mitochondrial beta-oxidation systems in animals causes severe biochemical abnormalities and clinical symptoms, it is likely that loss of the major system of beta-oxidation in plants would have severe consequences as well. There are four major enzymatic activities involved in the process of fatty acid oxidation in plants: acyl-CoA oxidase, enoyl-CoA hydratase, 3-hydroxacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase. There are several other enzymatic activities that are also necessary for the degradation of unsaturated fatty acids: [Delta]^3, [Delta]^2-enoyl-CoA isomerase, D-3-hydroxyacyl-CoA dehydratase, D-3-hydroxyacyl-CoA epimerase and 2,4-dienoyl-CoA reductase.

The first committed step in beta-oxidation is catalyzed by acyl-CoA oxidase (ACOX; EC 1.3.3.6) which converts fatty acyl-CoA into 2-*trans*-enoyl-CoA (Figure 1.2 A). ACOX is a unique marker enzyme of non-mitochondrial beta-oxidation in eukaryotic cells (Kunau et al., 1995). There are families of ACOXs in nearly all eukaryotic organisms, including plants. Three ACOXs have been purified from plants: short-chain (SCOX), medium-chain (MCOX) and long-chain (LCOX). ACOXs are soluble proteins of variable size and structure. Hooks et al. (1996) purified SCOX and MCOX from maize seedlings. The MCOX is a 62 kDa monomeric protein with a specificity for C_{10} to C_{14}-CoA. SCOX is a 15 kDa protein that functions as a homotetramer (60 kDa), with a specificity for C_4 to C_8-CoA. LCOX activity was purified as well and showed similar substrate specificity as a previously purified LCOX from *Cucumis sativis* (Kirsch et al., 1986). LCOX functions as a homodimer with subunits of 72 kDa. Plant LCOXs show a specificity for C_{14}-CoA and longer substrates with a peak specificity for C_{16}-CoA.

Previous studies in plants suggest that ACOX may be the primary step in controlling the flux of metabolites through the beta-oxidation pathway. Holtman et al. (1994) demonstrated that the addition of purified yeast ACOX to plant tissue extracts was sufficient to increase production of acetyl-CoA from long-chain acyl-CoAs, suggesting that ACOX was the rate limiting step in the reaction. An examination of the available ESTs in the Arabidopsis EST database reveals that there are at least 2 ACOX genes in Arabidopsis (see Table 1.1). The ACOX represented by the genomic clone, and its 8 corresponding ESTs, is likely to encode LCOX, based on its similarity to palmitoyl-CoA (C_{16}-CoA) oxidase and the size of the predicted protein (73.9 kDa). The 6 ESTs of group 2 probably represent another LCOX, based on their match to the ACOX from *Phalaenopsis*, which has the predicted size of an LCOX. There are no SCOX or MCOX from plants represented in the DNA or protein databases, so there may be ACOXs of these types already sequenced from Arabidopsis but no way to identify them.

Enoyl-CoA hydratase (ECH; EC 4.2.1.17) and 3-hydroxyacyl-CoA dehydrogenase (HDH; EC 1.1.1.35) are the second and third enzymes in the beta-oxidation pathway. ECH catalyzes the hydration of 2-*trans*-enoyl-CoA into 3-hydroxyacyl-CoA (Figure 1.2 B) and HDH catalyzes the reduction of 3-hydroxyacyl-CoA to 3-oxoacyl-CoA (Figure 1.2 D).

Figure 1.2 Reactions catalyzed by beta-oxidation enzymes

(A) Acyl-CoA oxidase (ACOX) catalyzes the first reaction of beta-oxidation, converting acyl-CoA esters to the corresponding 2-*trans*-enoyl-CoA esters. ACOX transfers electrons directly to oxygen, producing hydrogen peroxide as a result.

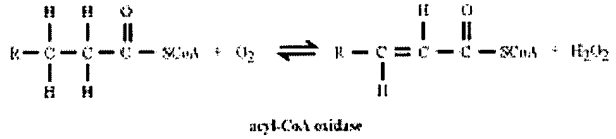
(B) Enoyl-CoA hydratase (ECH) catalyzes the second step of beta-oxidation, hydrating 2-*trans*-enoyl-CoA esters to 3-hydroxyacyl-CoA esters.

(C) [Delta]^3, [Delta]^2-enoyl-CoA isomerase (ECI) is required for the metabolism of *cis*-unsaturated fatty acids. ECI converts 3-*cis*-enoyl-CoA into 2-*trans*-enoyl-CoA, which then is acted on by ECH.

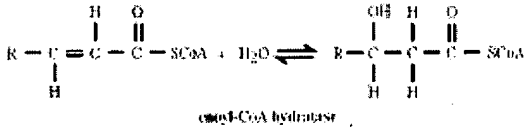
(D) L-3-hydroxyacyl-CoA dehydrogenase (HDH) catalyzes the third reaction in beta-oxidation, the oxidation of 3-hydroxyacyl-CoAs to 3-oxoacyl-CoAs. ECH and HDH are found together on multifunctional proteins in peroxisomal systems of beta-oxidation, often with ECI as well.
(E) The final reaction of beta-oxidation is catalyzed by 3-ketoacyl-CoA thiolase. 3-ketoacyl-CoAs are thiolytically cleaved to yield acetyl-CoA and an acyl-CoA that has been chain-shortened by two carbon units.

Figure 1.2 Reactions catalyzed by beta-oxidation enzymes

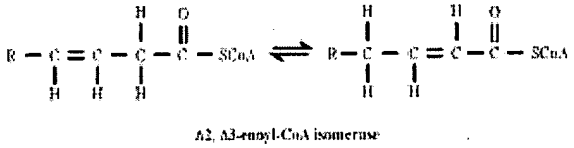
A



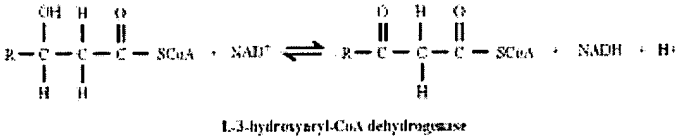
B



C



D



E

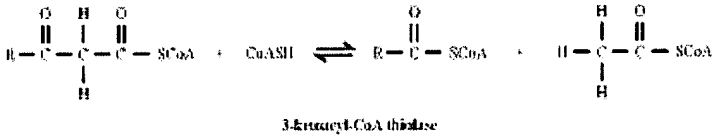


Table 1.1 Acyl-CoA oxidases and 3-ketoacyl thiolases in Arabidopsis

Databases searches were performed using the BLAST server at:
http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-blast?Jform=0
and an Arabidopsis specific sequence database at:
http://genome-www2.stanford.edu/cgi-bin/AtDB/nph-blast2atdb
Keyword searches were done at the following WWW sites:
The Institute for Genomic Research (TIGR)
http://www.tigr.org/tdb/at/atgenome/atgenome.html
Kazusa DNA Research Institute, Japan
http://www.kazusa.or.jp/arabi/
Munich Information Centre for Protein Sequences
http://www.mips.biochem.mpg.de/mips/athaliana/
The UK Crop BioInformatics Network, UK CropNet
http://synteny.nott.ac.uk/db.html
Expressed sequence tags (ESTs) and genomic sequences were placed into groups based on DNA

alignments using the BLAST server and information from the TIGR EST assembly database.

Table 1.1 Acyl-CoA oxidases and 3-ketoacyl thiolases in Arabidopsis

Acyl-CoA oxidase	
Group 1	(J02752) acyl-CoA oxidase [*Rattus norvegicus*]
T20673, T21971, T42499, T45531, N37687, N38332, T04472, W43736	Arabidopsis genomic sequence (Z97341); Chromosome 4
Group 2	(U66299) acyl-CoA oxidase [*Phalaenopsis* sp. 'True Lady']
T43584, AA042344, T04810, T45187, F13898, W43479	
3-Ketoacyl Thiolase	
Group 1	(X93015) glyoxysomal beta-ketoacyl-thiolase [*Brassica napus*]
T45659, Z18059, R65341, H37590, R89952, N37839, N65908, W43093, T04395, Z32626, Z35397, Z35342, Z37598, Z47692, T20943, T21319, T12940, Z32625, Z34664, N38511, Z18059, Z26868	Arabidopsis genomic sequence (AC002334); Chromosome 1
Group 2	(X93015) glyoxysomal beta-ketoacyl-thiolase [*Brassica napus*]
AC002376	Arabidopsis genomic sequence; Chromosome 2; no corresponding ESTs

Group 3	(X67696) acetyl-CoA acyltransferase [*Cucumis sativus*]
T75842	
Group 4	(X78116) acetoacetyl-CoA thiolase [*Raphanus sativus*]
T44571, T45052, H36507, F13985, F14053, H37662, AA404882	no DNA similarity to Group 5
Group 5	(X78116) acetoacetyl-CoA thiolase [*Raphanus sativus*]
T76117	no DNA similarity to Group 4
Group 6	(X78116) acetoacetyl-CoA thiolase [*Raphanus sativus*]
T76573	no DNA similarity to Group 7, may be 5' end of Group 4 or 5
Group 7	(X78116) acetoacetyl-CoA thiolase [*Raphanus sativus*]
N37718	no DNA similarity to Group 6, may be 5' end of Group 4 or 5

They constitute two of the enzymatic activities found on plant peroxisomal multifunctional proteins (MFPs).
In cucumber, a total of four multifunctional proteins have been purified and characterized (Behrends et al., 1988; Gühnemann-Schäfer and Kindl, 1995ab). These proteins are soluble proteins and range in size from 74 kDa to 81 kDa. Three of these proteins have been isolated from cotyledons (MFP I, II, III), while the fourth (MFP IV) was isolated from leaf tissue. The enzymatic properties of these four proteins differ; MFP I and MFP IV have no isomerase activity, indicating that they are trifunctional enzymes as opposed to the tetrafunctional MFP II and MFP III. In addition, the hydratase activity of these four proteins shows

chain-length specificity; MFP II shows no activity towards $C_{18:1}$ while MFP IV shows a higher activity toward shorter chain FAs (Gühnemann-Schäfer and Kindl, 1995a). No data is available for the chain-length specificity of the remainder of the enzymatic activities, though presumably they will show varying specificity as well. Another MFP, designated MFP-b, has been cloned and shown to be a tetrafunctional protein, and may represent a fifth member of the cucumber MFP family (Preisig-Müller et al., 1994). By expressing portions of this protein in *E. coli*, the authors were able to assign enzymatic activities to specific protein domains. A bifunctional enzyme of 75 kDa, containing ECH and HCDH, has also been purified from pea seedlings (Miernyk et al., 1991). No test was made for ECI or epimerase activity though, so the enzyme purified may represent a member of the MFP family as well.

Table 1.2 Multifunctional proteins, acyl-CoA dehydrogenases and enoyl-CoA hydratases in Arabidopsis

Multifunctional Protein	
Group 1	*AIM1*
Z31666, Z33957	
Group2	*AtMFP2*
N96406, T45936, T75974	
L-3-hydroxyacyl-CoA dehydrogenase	
Group 1	(U32229) HbdA [*Bradyrhizobium japonicum*]
Z34008, T76048	may be 5' end of group 2
Group 2	(U32229) HbdA [*Bradyrhizobium japonicum*]
T04112, Z34009	may be 3' end of group 1
Acyl-CoA dehydrogenase	

Group 1	(Z66513) glutaryl-CoA dehydrogenase (F54D5.7) [*Caenorhabditis elegans*]
U72505, H77217	
Enoyl-CoA hydratase	
Group 1	(U08976) peroxisomal enoyl hydratase-like protein [*Rattus norvegicus*]
T42856, T43247	
Group 2	(X79888) AU-binding protein/Enoyl-CoA hydratase [*Homo sapiens*]
Z97342	Arabidopsis genomic sequence; Chromosome 4; no corresponding ESTs
Group 3	(U17110) crotonase [*Clostridium acetobutylicum*]
N97282, Z97340	Arabidopsis genomic sequence; Chromosome 4
Group 4	(X73904) carnitine racemase [*Escherichia coli*]
AC001229	Arabidopsis genomic sequence; Chromosome 4; no corresponding ESTs
Group 5	(X73904) carnitine racemase [*Escherichia coli*]

Z97336	(e326890) Arabidopsis genomic sequence; Chromosome 4; no corresponding ESTs
Group 6	(X73904) carnitine racemase [*Escherichia coli*]
Z97336	(e327452) Arabidopsis genomic sequence; Chromosome 4; no corresponding ESTs

Database searches and keyword searches were conducted as described for Table 1.1

MFPs also contain [Delta]^3, [Delta]^2-enoyl-CoA isomerase (ECI; EC 5.3.3.8) and may also contain 3-hydroxyacyl-CoA epimerase. ECI shifts the 3-*cis* double bond of the intermediates of unsaturated fatty acid oxidation to the 2-*trans* position (Figure 1.2 C). A monofunctional ECI, that acts as a homodimer, with subunit size of 24 kDa, has been purified from cucumber (Engeland and Kindl, 1991b). This enzyme was active with 3-enoyl-CoA species ranging from C_6 to C_{12} with *cis*-hexenoyl-CoA being the most effective substrate. There are monofunctional ECIs in other organisms, mainly mammals, that are mitochondrial proteins approximately 32 kDa in size. To date, there is no indication of monofunctional ECI proteins in the Arabidopsis based on database searches, but the difference in size between the cucumber ECI and the mammalian mitochondrial ECI may mean that they share little protein similarity. Examination of Arabidopsis cDNA and genomic sequence indicate there may be a number of monofunctional enoyl-CoA hydratases and at least one monofunctional 3-hydroxyacyl-CoA dehydrogenase in the Arabidopsis genome (see Table 1.2). Several of the ECHs may represent mitochondrial enzymes involved in beta-oxidation, based on their size, which is similar to that of monofunctional ECHs, and lack of peroxisomal targeting signal (discussed in Chapter 5). Two monofunctional D-2-*trans*-enoyl-CoA hydratases have been purified from cucumber cotyledons (Engeland and Kindl, 1991a). These hydratases act as homodimers with subunits of approximately 33 kDa. Several of these groups, then, may represent these other forms of hydratases. Group 2 represents a 24 kDa protein, small in comparison to other known peroxisomal ECHs. Groups 4, 5 and 6 in Table 1.2 show similarity to carnitine racemase, that in turn shows similarity to enoyl-CoA hydratases/isomerase (Eichler et al., 1994). Carnitine racemaces function in carnitine metabolism in *E. coli* (Eichler et al., 1994), and L-carnitine plays a major role in eukaryotes in transporting fatty acids into the mitochondria (Kunau et al., 1995). Group 5 and group 6 lie immediately adjacent to one another on chromosome 4 and thus may represent a gene duplication event. Neither protein has an established peroxisomal targeting signal, suggesting that they may not be involved in peroxisomal beta-oxidation, but may be involved in mitochondrial beta-oxidation. Group 4 is very similar to these two proteins and also lies on chromosome 4, but unlike them, it contains a peroxisomal targeting signal at the carboxy terminus, -SKL.

The final step of beta-oxidation is catalyzed by 3-ketoacyl-CoA thiolase (EC 2.3.1.16). Thiolase catalyzes the cleavage of 3-oxoacyl-CoA (C_n) to acyl-CoA (C_{n-2}) and acetyl-CoA (Figure 1.2 E). Thiolase has been cloned from cucumber (Preisig-Müller and Kindl, 1993), mango (Bojorquez and Gómez-Lim, 1995), pumpkin (Kato et al., 1996) and *Brassica napus* (Olesen et al, 1997). They are soluble proteins ranging in size from 45 to 49 kDa and each contains a cleavable amino-terminal signal peptide (PTS2, discussed in Chapter 5) directing the protein to the peroxisome. There are at least five 3-ketoacyl thiolase or acetoacetyl-CoA thiolase genes in Arabidopsis. Groups 1, 2 and 3 in Table 1.1 are likely to represent the thiolases responsible for acting on long-chain fatty acid chains. Groups 4 through 7 likely represent two

genes that act only on acetoacetyl-CoA. They may be mitochondrial proteins or peroxisomal (Kunau et al, 1995). One interesting point is that though two thiolase genes have been found by genomic sequencing (Groups 1 and 2), only one of them has ESTs corresponding to it. In fact, 22 out 34 of the ESTs found in the database correspond to the locus on Chromosome 1. The second thiolase gene, found on chromosome 2, may not be represented in the cDNA libraries examined because it is expressed only under certain conditions, or perhaps because it is a senescence-associated gene that is only expressed late in development. These types of genes are often poorly represented in cDNA libraries made from green, healthy tissue.

Most the work done on beta-oxidation enzymes has involved the characterization of their biochemical and kinetic properties after isolation and purification from plant tissues, usually fatty tissues. Until recently, there were no known mutations in plant beta-oxidation enzymes. Loss of any of these enzymatic activities might be expected to cause developmental phenotypes. However, one would predict that germination and seedling development would be the most affected by the loss of the beta-oxidation system. beta-oxidation in the glyoxysomes of germinating seedlings is responsible for mobilizing seed lipid reserves and channeling the resulting acetyl-CoA into gluconeogenesis via the glyoxylate cycle (Gerhardt, 1992). Indeed, there are two examples of deficiencies in beta-oxidation that affect seed germination. Acyl-CoA oxidase antisense plants show a sucrose dependent phenotype for germination (Ian Graham, personal communication). Seedlings grown on MS media minus sucrose show cotyledon emergence but subsequent growth is stalled. There is no apparent phenotype in the adult plant.

Hayashi et al. (1997) have devised a selection scheme for beta-oxidation mutants utilizing the herbicide 2,4-DB (see Chapter 3). Four of the twelve mutants they isolated show sucrose-dependent germination, like the ACOX antisense plants. This emphasizes the importance of beta-oxidation in mobilizing seed lipid reserves in germinating seedlings. The lack of an adult phenotype in any of these mutants suggests that there are separate systems for mobilizing lipid reserves and for normal house-keeping function and/or senescence-associated activities.